METHODS FOR TESTING EFFECTS OF FUNGISTATIC COMPOUNDS IN LACQUER*

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Most dermatomycoses and particularly onychomycosis are often very resistant to therapy. Moreover, successful treatment of the latter infection becomes more important because of its high incidence. White (1), for example, has considered 20 per cent of all nail disturbances to be of fungus origin. As a method of treatment for onychomycosis Rothman (2) and Langer and Kaden (3) have used nail lacquer containing antimycotic substances. Further investigation of certain aspects of the inhibitory effect of antimycotic lacquers is presented in this paper.

MATERIALS AND METHODS

An antifungal agent, halogenocyclohexane[1] was added to a nitrocellulose finger nail lacquer in a final concentration of 2 per cent. The anti-fungal effect of this compound in lacquer (Preparation ✗ 304) was compared with a lithium bromide-Asterol® base lacquer, (Ro), proposed by Rothman (2) and with a common nail lacquer (B) to which no fungistatic agent had been added. The fungi used in the various tests included *Penicillium glaucum*, *Aspergillus fumigatus*, *Trichophyton rubrum*, *Microsporum Audouini*, *Epidermophyton floccosum*, and *Candida albicans*.

Three test methods were used to demonstrate the effect of the antimycotic lacquers.

(1) Slide film method: This method was described by Kaden (4). A strip of lacquer film is applied to the width of a sterile microslide and allowed to dry for two hours. Molten Sabouraud's glucose agar was inoculated with the fungus to be tested and, with a sterile pipette, two strips of this agar was run the length of the prepared slides. The slides were placed in Petri dishes for incubation at room temperature. Inhibition was determined by the extent of clear zones, in mm., from the lacquer strips.

(2) Disc Method: This method was based on that used by Ruggeri (1950) (5) for testing the fungicidal activity of varnishes and lacquers. A Petri dish of Sabouraud's glucose agar was inoculated evenly over the entire surface with a suspension of the fungus to be tested. Lacquer samples were painted on one side of ⅞" cover glasses which were used as discs. The lacquer film was allowed to dry for two hours after which the covers were placed lacquer side up on the agar surface. The Petri dish cultures were incubated at room temperature and observed for a period of ten days. Inhibition of growth was determined by the size of the inhibitory zone, in mm., around the covers. Inhibition under the covers was noted also and termed an additional effect.

(3) Evaporation test: Petri dishes were filled with enough Sabouraud's glucose agar (approx. 60 ml) to leave a 5 mm. space between the agar surface and the cover. The agar

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* The experimental investigation has been completed and the paper was written during a Fulbright Fellowship, spent with Dr. Norman F. Conant, Professor of Mycology, Department of Bacteriology, Duke University School of Medicine, Durham, North Carolina.

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Received for publication August 8, 1955.

[1] Antimycotic-Labopharma, Labopharma, Berlin, Germany.

surface was inoculated evenly with a suspension of the fungus to be tested. The entire inside of another Petri dish cover was painted with lacquer which was allowed to dry for two hours. This lacquered cover was placed on the test Petri dish. The results of inhibition, by continuing vaporization, was determined after an incubation period of four to ten days at room temperature.

RESULTS

Nail lacquer preparation ✱304 showed good inhibitory activity against a variety of fungi in the three tests described above. In the slide film test this lacquer suppressed growth of *Penicillium glaucum*, during a seven days incubation period for a distance of 6.5 mm. along the agar strip. Under the same conditions a similar effect was obtained with a nail lacquer (Ro) containing lithium bromide and Asterol®. Growth of the fungus in agar strips was not inhibited on slides "striped" with common nail lacquer (B) and on control (C) slides (figure 1).

The inhibitory effect of preparation ✱304 against *Aspergillus fumigatus* is demonstrated in figure 2. In this figure will be seen a wide zone of inhibition surrounding the cover glass previously painted with lacquer containing the antifungal agent. Fungus growth was also inhibited for some distance beneath this cover glass. No inhibition was noted around the cover glass painted with common lacquer or in the area covered by a clean unpainted cover glass.

The effect of preparation ✱304 on the growth of a variety of fungi using the "disc" method is shown in Table I. In this table it can be seen that the growth of *T. rubrum*, *M. Audouini* and *E. floccosum* was markedly inhibited in the presence of preparation ✱304.

Complete inhibition of *M. Audouini*, *T. rubrum*, *E. floccosum*, *C. albicans* and

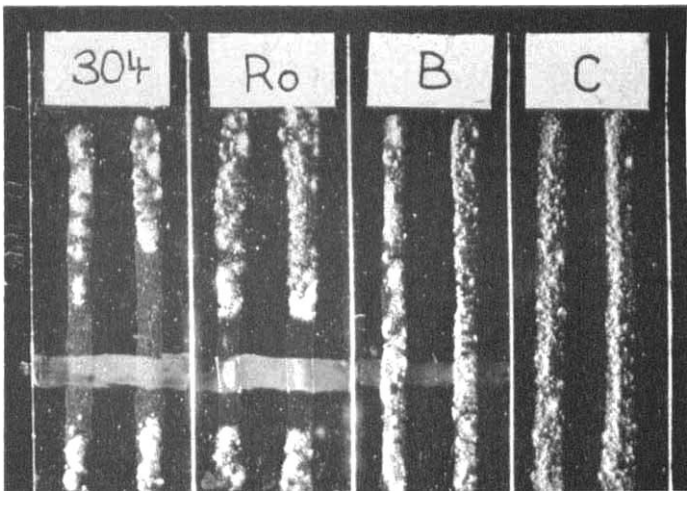

FIG. 1. Slide film test method. *Penicillium glaucum*, 7 days incubation. ✱304 Halogenocyclohexane in lacquer; Ro Lithium bromide Asterol® in lacquer; B. Lacquer only; C. Control slide.

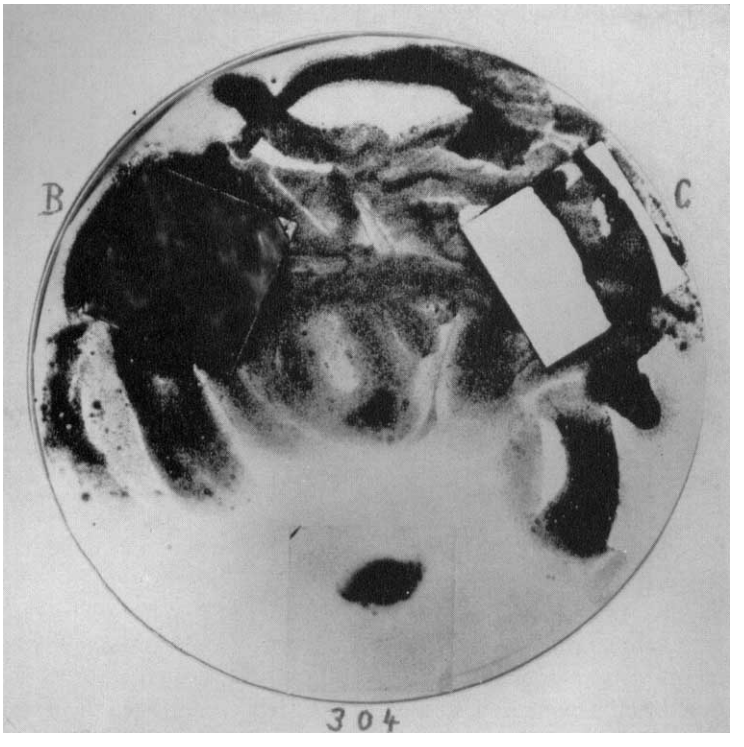

FIG. 2. Disc test method. *Aspergillus fumigatus*, 5 days incubation showing inhibition of #304 Halogenocyclohexane in lacquer; B. Lacquer only; C. Control disc. Note inhibition under cover glass "disc" in 304.

TABLE I

Zone of inhibition in millimeters in the areas adjoining the discs

Test fungus	Samples applied to the discs (⅞" cover glasses)		
	Preparation 304	Common nail lacquer	Control
Trichophyton rubrum	15	0	0
Microsporum Audouini	14	0	0
Epidermophyton floccosum	20	0	0
Aspergillus fumigatus	8	0	0
Candida albicans	3	0	0

A. fumigatus was observed in Petri dishes when the inside of the cover had been painted with preparation #304. This effect is illustrated in figure 3. In figure 3 it can be seen that *M. Audouini* failed to grow when the inoculated Petri dish culture was covered with a cover painted on the inside with the antifungal lacquer. Growth was not inhibited in the control culture or in the culture covered with common nail lacquer. Growth of common contaminants such as *Penicillium* sp., *Candida* sp. and bacteria were also inhibited in this test.

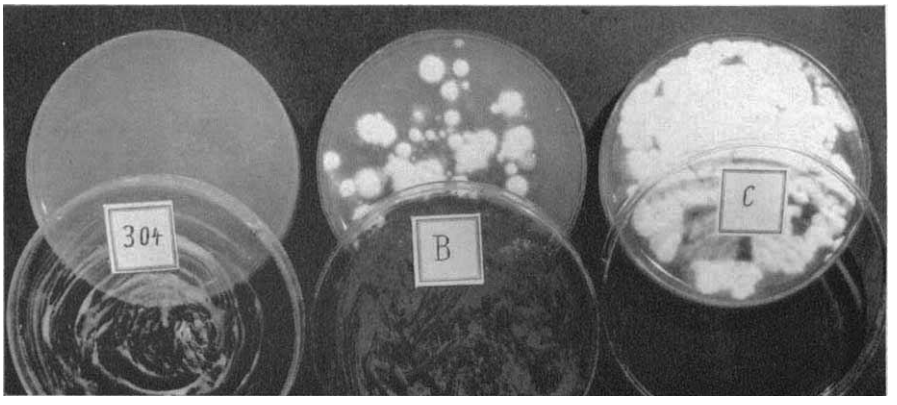

FIG. 3. Evaporation test. *Microsporum Audouini*, 7 days incubation. ✱304 Under surface of cover painted with Halogenocyclohexane lacquer; B. Painted with lacquer only; C. Control.

DISCUSSION

The halogenocyclohexane derivative used in the experiments described above is chemically related to some of the known effective fungistatic agents. In the United States, Ruggeri (5) mentioned pentachlorphenol, in Germany, Rieth (6) tested isomeres of hexachlorcyclohexane and in France, Nedey (7) described tri-, tetra-, and pentachlorphenol as fungicidal agents. Attempts to incorporate these chemicals in nail lacquer, however, have not proved successful.

Hexachlorcyclohexane and its derivatives was shown to act against insects, bacteria or fungi depending on a single change in their isomere structure (Czyzewski (8) and Green (9). One of its derivatives, halogenocyclohexane, was shown to have good fungistatic properties and it was also found to be easily incorporated into a nitrocellulose nail lacquer. Its fungistatic effect is dependent on the slow evaporation of the compound from the lacquer base. It can be used to inhibit immediately spore germination or, as Grimmer (10) has shown, it can be used to inhibit further growth of a culture after varying periods of incubation. Cultures thus treated can be used for teaching and demonstration purposes. In addition to the laboratory tests with so-called fungistatic lacquers such materials have also been used in therapeutic tests.

Rothman (2) reported good results in the treatment of onychomycosis with an antimycotic lacquer containing lithium bromide and Asterol®. However, poultices soaked in lithium bromide solution and special sprays were added to the therapeutic scheme. Kaden (3), using only halogenocyclohexane in lacquer (Preparation ✱304)[2] reported satisfactory results in the treatment of skin and nail infections. Meyer (11) also reported successful clinical trials with the halogenocyclohexane derivative in mycotic infections of the skin.

[2] Preparation ✱304 is identical with "Mykotektan", Labopharma, Berlin, Germany and "Tektopal . . .", Philadelphia Ampoule Laboratories, Philadelphia, Pennsylvania.

CONCLUSION

Halogenocyclohexane derivative, incorporated in a nitrocellulose nail lacquer showed marked inhibitory effects against *M. Audouini*, *T. rubrum*, *E. floccosum*, *C. albicans*, *Penicillium glaucum* and *Aspergillus fumigatus*. The fungistatic activity was due to a permanent evaporation of the effective agent as shown by three *in vitro* test methods.

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